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Award Number: W81XWH-10-1-0222

TITLE: Toward Personalized Ovarian Cancer Therapy through The Cancer Genome Atlas

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REPORT DATE: April 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
April 2011	Annual	1 April 2010 – 31 March 2011

4. TITLE AND SUBTITLE

Toward Personalized Ovarian Cancer Therapy through The Cancer Genome Atlas

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0222

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Sloan-Kettering Institute for Cancer Research
New York, NY 10065

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

There is a growing recognition that the molecular biology of ovarian cancers is complex and that the disease is correspondingly heterogeneous. There is a need for platforms that can translate molecular findings into useful diagnostic tests for patient stratification, therapeutic decision making, and clinical trial design. The Cancer Genome Atlas (TCGA) pilot project is comprehensively cataloguing the genomic aberrations of advanced serous ovarian carcinoma. The project objective is to identify proteins and corresponding signaling pathways that (1) correlate with the genomic alterations in ovarian carcinoma as determined by TCGA and (2) can be used as biomarkers for clinical outcome and/or chemotherapy response. We have optimized 44 IHC assays for ovarian cancer tissues. We have collected more than 150 TCGA qualified tumors and created Tissue MicroArrays (TMAs). We have incorporated modified and updated TCGA findings into our study design. Antibodies to drug targets or markers of drug activity have been selected and additional pathway components and markers have been added based on findings from TCGA. The panel will focuses on the most relevant and novel signaling pathways for serous ovarian carcinoma.

15. SUBJECT TERMS
Ovarian Carcinoma, The Cancer Genome Atlas, Immunohistochemistry

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

There is a growing recognition that the molecular biology of ovarian cancers is complex and that the disease is correspondingly heterogeneous. There is a need for platforms that can translate molecular findings into useful diagnostic tests for patient stratification, therapeutic decision making, and clinical trial design. The Cancer Genome Atlas (TCGA) pilot project is comprehensively cataloguing the genomic aberrations of advanced serous ovarian carcinoma. The project objective is to identify proteins and corresponding signaling pathways that (1) correlate with the genomic alterations in ovarian carcinoma as determined by TCGA and (2) can be used as biomarkers for clinical outcome and/or chemotherapy response.

BODY:

Task 1: To select and optimize IHC assays for a Tumor Marker panel that will be preformed in CLIA-certified labs.

1a. Approximately 40 markers for inclusion in the Tumor Marker panel will be identified based on knowledge of targeted drugs available or in development and through review of activated pathways identified by TCGA that correlate with tumor classification. (3 months)

Progress: We have tested 44 antibodies in a panel of ovarian cancer tissues (See Table 1, supporting data). Of these 44 markers, 3 antibodies failed, and 13 were uninformative due to uniformity across all tissues, leaving 28 informative antibodies which will be taken forward into our tumor marker panel. Due to a number of uninformative markers we are continuing to obtain additional markers relevant to the research goals.

1b. Antibodies to markers selected in 1a will be divided into those that are currently in use through CLIA-approved laboratories and those that have commercially available antibodies but are not currently in use at CLIA-approved laboratories.

1b1. For antibodies in use, we will identify appropriate laboratories and establish contractual agreements for staining and interpretation of markers. We will work with Clarient, Inc as one CLIA-certified laboratory because we have a relationship with them, they have many of the proposed markers in clinical use, and have already generated data from some TCGA samples are part of our preliminary data. For newly developed markers and markers not in use at Clarient, we will identify other CLIA-certified laboratories that can meet the project requirements (3 months)

Progress: We have identified numerous commercially available antibodies. However, most of these antibodies are primarily used in research applications. Thus we have been unable to have these antibodies run in CLIA-certified laboratories. The antibodies have all been run in facilities that have CLIA-certified laboratories, but since the antibodies are currently not in clinical use, for the most part, we have had to run them in research laboratories associated with CLIA-certified laboratories.

1b2. For antibodies not in use, we will optimize and validate antibodies at CLIA-approved or academic laboratories. If optimized at an academic laboratory, validation will be conducted at a CLIA-approved laboratory. The academic laboratory optimization will take place in the Memorial Sloan-Kettering Core Immunohistochemistry Research Laboratory which has a long history of optimizing the methodology for the application of monoclonal and polyclonal antibodies for use on human tissues and for assessing the tissue distribution of antigens. (6 months)

Progress: We have optimized a number of previously unvalidated antibodies including antibodies to BRCA1, PTEN, c11orf30, MUC16. We have been unsuccessful optimizing antibodies to BRCA2 and

SOX11. We will not be able to move all these antibodies into CLIA-certified laboratories, but we are in the process of moving PTEN into a CLIA-certified laboratory and we have previously moved MUC16 into a CLIA-certified laboratory. We will soon plan on moving BRCA1 into a CLIA-certified laboratory after additional validation.

Task 2: To profile TCGA-qualified tumors for expression of proteins in the Tumor Marker panel
2a. Create multiple TMAs for TCGA-qualified tumors

2a1. Establish material transfer agreements for each tissue source site – currently 4 – and complete regulatory (IRB) requirements at each tissue source site as necessary. (3 months)
Progress: We have established MTA and IRB agreements with Cedars-Sinai, Duke University, Mayo Clinic. We have been unsuccessful to date working with Washington University – St. Louis. We have ongoing discussions with UCSF.

2a2. Pull tissue blocks from TCGA-qualified tumors and create TMA(s) for included specimens. At least 200 patient samples will be included in the TMAs. However, it is likely that the 4 tissue source sites and others will contribute a total of 250 qualified samples by the time the funding period begins. We will include all available samples at the time of TMA creation. This will occur separately at each of the 4 tissue source sites and additional sites as indicated above. Cedars-Sinai is the recipient of a subaward of this proposal. Washington University and Mayo Clinic have both agreed to create TMAs for use in this project and letters of collaboration are attached. (3 months)
Progress: We have created TMAs from MSKCC, Cedars-Sinai, Duke University, and Mayo Clinic samples for a total of 225 TCGA samples. We have had trouble obtaining samples from Washington University despite having a pre-award letter of collaboration. We have ongoing discussions with UCSF.
2b. Stain and score all TMAs for antibodies in Tumor Marker panel at CLIA-approved laboratories. (6 months)
Progress: We have begun to stain and score the TMAs. This is an ongoing process.

Task 3: To correlate protein expression profiles with tumor classification from TCGA analyses.
3a. Identify genomic sub-classes of TCGA tumor samples. This will be performed by TCGA and the MSKCC TCGA genomic characterization center will assist us in obtaining this data as necessary – see letter of collaboration. (3 months)
Progress: We have identified markers representative of the 4 transcriptome tumor subtypes reported by TCGA. We have modified the markers during this reporting period (Year 1) as the TCGA ovarian cancer manuscript was just published in Nature in June of 2011, a bit later than had been anticipated for unclear reasons. We have also identified markers representative of other major genomic findings from TCGA. We have markers representative of copy number alterations and frequently mutated genes.
3b. Correlate tumor subclasses with expression of proteins in tumor marker panel and with clinical outcomes and identify refined protein marker panel. This will be performed by our biostatistician from MSKCC (4-6 months)
Progress: Pending

Task 4: To validate the established classifiers.
4a. Pull tissue blocks from validation tumor set and create validation TMA(s). This will be performed at each of the 4 tissue source sites. (3 months)
Progress: We have identified 100 validation cases from MSKCC.

4b. Stain validation TMAs for an tibodies in protein classifier at CLIA-approved laboratories. (3 months)
4c. Determine predictive accuracy of validation samples and protein classifier. This will be performed by our biostatistician at MSKCC. (3 months)
4d. Publication of findings. The PI will work with the other investigators to generate a manuscript for publication. (3 months)
Progress: The remainder of Task 4 is pending.

KEY RESEARCH ACCOMPLISHMENTS:

- Antibodies for tumor marker panel have been chosen and optimized.
- TMAs for TCGA qualifying samples have been created.
- TMA staining and scoring has been partially completed and is ongoing.

REPORTABLE OUTCOMES:

A collection of TMAs based on TCGA-qualified cases have been created. This tissue repository has been offered as a resource to other appropriate investigators. An active DOD grant application for a Translational Leverage Award by an independent research group has included this resource in their application as an existing source of useful and important biospecimens.

CONCLUSION:

It is somewhat premature to make definitive conclusion regarding the implications of the completed research as the work is very much in progress at the end of Year 1. We have had trouble obtaining samples from all sites, but will meet our proposed target goal. Scoring of TMAs is ongoing and this is proving to be a very time consuming aspect of the project as we had anticipated. To date, we have produced TMAs from TCGA samples which are a very valuable resource. In fact, the NCI has recently launched phase II of the Clinical Proteomics Tumor Analysis Consortium, a 50 million dollar effort. One of the main goals is to perform proteomic profiling of TCGA qualified cases. Ovarian cancer will feature prominently in this effort and the biospecimens collected as part of this current proposal will also serve as a valuable resource.

REFERENCES: Not applicable.

APPENDICES: None

SUPPORTING DATA:

Table 1. Antibodies tested

Protein	Comments	Status
AURKA	High background, poor localization	Failed Antibody
AURKB	Good staining	Include
BCL2	All negative	Uninformative
BIRC5	Good staining	Include
BRCA1	Good staining	Include
CASP3	All weak	Uninformative
CCND1	Good staining	Include
CCNE1	Good staining	Include
CDH1	All positive	Uninformative
CDKN1A	Good staining	Include
CDKN1B	Good staining	Include
CDKN2A	Good staining	Include
Cl. CASP3	Good staining	Include
CTNNB1	Good staining	Include
EGFR	Good staining	Include
EIF4EBP1	All positive	Uninformative
ERCC1	Good staining	Include
FOXP3	All negative	Uninformative
HIF1A	Good staining	Include
IGF1R	Good staining	Include
ITGA4	All negative	Uninformative
MKI67	Good staining	Include
MSLN	Good staining	Include
MTOR	All positive	Uninformative
MUC16	Good staining	Include
MYC	Good staining	Include
NF2	All weak	Uninformative
NFKB1	All positive	Uninformative
NFKBIA	Poor localization	Failed Antibody
NOTCH1	Good staining	Include
p-AKT1	Good staining	Include
PARP1	All positive	Uninformative
p-EIF4E	All weak	Uninformative
p-EIF4EBP1	Good staining	Include
p-MAPK1	Good staining	Include
p-MTOR	Good staining	Include
p-PDGFRA	All positive	Uninformative
PPP1R15A	Poor localization	Failed Antibody
p-RPS6	Good staining	Include
PTEN	Good staining	Include
RAD51	Good staining	Include
RB1	Good staining	Include
TGFB3	All positive	Uninformative
TP53	Good staining	Include